Human Papillomavirus Testing Following Loop Electrosurgical Excision Procedure Identifies Women at Risk for Posttreatment Cervical Intraepithelial Neoplasia Grade 2 or 3 Disease

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Abstract

Background: **Loop electrosurgical excision procedure (LEEP) is the predominant treatment for cervical intraepithelial neoplasia grade 2 or 3 (CIN2+) in the United States, yet following treatment ~10% of women are diagnosed again with CIN2+, necessitating close follow-up of such patients.**
Methods: **Surveillance strategies using cytology and/or human papillomavirus (HPV) testing were compared among women who underwent LEEP (n = 610) in the Atypical Squamous Cells of Undetermined Significance (ASCUS) Low-Grade Squamous Intraepithelial Lesion (LSIL) Triage Study. Cervical specimens, collected at 6-month visits for 2 years, were used for cytology, Hybrid Capture 2 (HC2) detection of carcinogenic HPVs, and PCR for genotyping of carcinogenic and noncarcinogenic HPV types. At exit, women had colposcopy for safety and disease ascertainment.**
Results: **At the visit post-LEEP (median time: 4.5 months after LEEP), 36.9% [95% confidence interval (95% CI), 32.7-41.1%] of women were positive for carcinogenic HPV by PCR and 33.7% (95% CI, 29.7-37.9) had ASCUS or more severe (ASCUS+) cytology. The overall 2-year cumulative incidence of histologically confirmed posttreatment CIN2+ was 7.0%; this could be further stratified by the HPV risk category detected at the 6-month visit after LEEP. The 2-year risk associated with HPV16 positivity was 37.0%, significantly higher than for other carcinogenic HPV types (10.8%, $P < 0.001$), noncarcinogenic types (1.5%, $P < 0.001$), or testing HPV negative (0%). Post-LEEP cytology (using a positive threshold of ASCUS+) was 78.1% (95% CI, 60.0-90.7%) sensitive for detection of posttreatment CIN2+. By comparison, PCR for carcinogenic HPV and combination testing (using a positive result from carcinogenic HPV testing or cytology as the test threshold with HPV-negative ASCUS not referred) were significantly more sensitive (96.9% for each, $P = 0.03$); HC2 alone was nonsignificantly more sensitive (90.6%, $P = 0.3$). Specificity was similar for ASCUS+ cytology (69.1%, 95% CI, 64.6-73.3%) and PCR for carcinogenic HPV (67.1%, $P = 0.5$), yet was lower for HC2 (63.8%, $P = 0.048$) and combination testing (62.9%, $P = 0.02$).**
Conclusion: **Women who tested positive after LEEP for carcinogenic HPV types, especially HPV16, had high risk of subsequent CIN2+. HPV-based detection methods, alone or in combination with cytology, may be useful to incorporate in post-LEEP management strategies.** (Cancer Epidemiol Biomarkers Prev 2006;15(5):908–14)

Introduction

Since its introduction in 1989 (1), loop electrosurgical excision procedure (LEEP) has quickly become the most common cervical treatment modality in the United States for cervical intraepithelial neoplasia grade 2 or 3 (CIN2+). LEEP is highly effective and offers the following advantages: (*a*) unlike ablative techniques, LEEP provides a tissue specimen for histologic evaluation, and (*b*) compared with cold knife conization, LEEP removes less normal tissue (2, 3). However, following LEEP, ~10% of women have CIN2+ due to either residual or recurrent disease (2, 3).

In previous studies, risk of residual or recurrent disease has been consistently associated with large lesion size before LEEP, endocervical extension of disease, and incomplete excision of the lesion (4-7). However, even women with clear margins following excision are at risk for disease recurrence (8). Carcinogenic human papillomavirus (HPV), the causative agent of cervical cancer and its precursor lesions, is present in up to one third of women following LEEP and is associated with disease recurrence (5, 6, 9-11). Therefore, HPV testing may serve as a surveillance tool for identifying women at high risk of recurrence. Two meta-analyses that investigated the clinical utility of post-treatment carcinogenic HPV testing and cytology showed greater sensitivity of carcinogenic HPV testing compared with cytology for identifying recurrent/residual disease (12, 13). However, combination carcinogenic HPV testing and cytology (with a positive result from either test triaging to colposcopy) increased sensitivity further and was proposed as a method for monitoring women after treatment for CIN3 (12).

Due to the elevated risk of post-treatment CIN2+ in women who have had LEEP, maximizing test sensitivity is of greater importance than it would be in a screening population in which most subjects have relatively low risk of disease. In the

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United States, the current consensus guidelines for the management of women treated for CIN recommend follow-up by either cervical cytology at 4- to 6-month intervals with atypical squamous cells as the threshold for referral to colposcopy, or HPV DNA testing at least 6 months after treatment (14). Indefinite annual cytology follow-up is recommended to identify late recurrences (14). However, the guidelines acknowledge that this recommendation is based on a limited number of observational trials that defined the performance of various posttreatment surveillance methods. Therefore, we sought to evaluate and compare the clinical utility of cytologic assessment and HPV-based testing methods for identifying women at risk for posttreatment CIN2+ within the Atypical Squamous Cells of Undetermined Significance (ASCUS) Low-Grade Squamous Intraepithelial Lesion (LSIL) Triage Study (ALTS).

Materials and Methods

Overview. ALTS was a randomized controlled trial that compared three strategies for the initial management of equivocal and low-grade cytologic abnormalities. The methods have been previously described (15, 16). Briefly, women (n = 5,060) with a community-read cytologic interpretation of either ASCUS (n = 3,488) or LSIL (n = 1,572) were enrolled from January 1997 to December 1998 and followed for 2 years at four study sites [University of Alabama at Birmingham (Birmingham, AL), Magee-Women's Hospital of the University of Pittsburgh Medical Center Health System (Pittsburgh, PA), the University of Oklahoma (Oklahoma City, OK), and the University of Washington (Seattle, WA)]. Women were randomized into one of three management arms: immediate colposcopy, HPV triage, and conservative management. At enrollment, the arms differed in their referral criteria for colposcopy: In the immediate colposcopy arm, all women were referred; in the HPV triage arm, Hybrid Capture 2 (HC2; Digene Corporation, Gaithersburg, MD)–positive women, women with a missing HC2 result, or women with an enrollment cytologic interpretation of high-grade squamous intraepithelial lesion (HSIL) were referred; and in the conservative management arm, referral was based on a cytologic interpretation of HSIL. Nearly all colposcopy referrals in the HPV triage arm were related to positive HC2 tests. During follow-up, which consisted of reexamination every 6 months for 2 years regardless of study arm, women with a cytologic interpretation of HSIL were referred to colposcopy. Women who had a colposcopy-directed biopsy diagnosed as histologic CIN2 or more severe disease (CIN2+) were offered treatment by LEEP. All women attending the exit visit underwent colposcopy and those with persistent ASCUS associated with HPV infection, LSIL, or more severe lesions were treated by LEEP.

At each visit, nurse-clinicians conducted a pelvic examination and collected two cervical specimens. The first specimen was placed into PreservCyt (Cytyc Corporation, Marlborough, MA) for thin-layer liquid-based cytologic assessment and HC2 testing. A ThinPrep (Cytyc Corporation) slide was prepared and interpreted by local pathologists from the participating clinical centers. The second cervical specimen was placed in specimen transport medium (Digene Corporation) for type-specific HPV DNA testing. Following collection of these specimens, the cervix was rinsed twice with a 5% solution of acetic acid and the nurse-clinicians obtained two replicate cervigram photographs (National Testing Laboratory, Fenton, MO). An interviewer-administered questionnaire was used to query demographic characteristics (e.g., race and age), sexual behavior history (e.g., age at first intercourse and lifetime number of sexual partners), and potential cofactor information (e.g., oral contraceptive pill use, smoking, and parity).

HPV Testing. HPV DNA was assayed using two methods: HC2 and PCR-based detection of individual HPV genotypes (PCR). HC2 testing was done using an aliquot of the residual PreservCyt specimen according to a standard protocol. HC2 uses a pooled probe to detect 13 types considered to be carcinogenic (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) with 1.0 RLU/PC (~1 pg/mL) as the threshold for a positive result. A positive HC2 test result represents infection with one or more of the 13 types without identifying the specific type(s) detected. HPV testing using L1 PGMY09/11 PCR primers with line blot hybridization was done on cervical specimen transport medium samples to separately detect >27 genotypes, including the same 13 carcinogenic types targeted by HC2, as well as types considered possibly and noncarcinogenic (HPV6, 11, 26, 34, 40, 42, 53, 54, 55, 57, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73 [MM9], 81, 82 [MM4], 82V, 83 [MM7], 84 [MM8], CP6108; refs. 17, 18).

Cervicography. Cervigrams were processed according to the protocol of the manufacturer and interpreted in a blinded manner. For this study, results were categorized as ''technically defective'' if the cervigram could not be interpreted, ''negative'' if no abnormalities were found, ''atypical'' if the lesion was of doubtful significance, and ''P1+'' if a low-grade or more severe lesion was suspected. Cervigrams were selected for this analysis from three groups: all women with CIN2+ post-LEEP (n = 34); a random sample (n = 60) of women who had type-specific persistent HPV infection but no recurrence of CIN2+; and a random sample (n = 60) of women who cleared their HPV infection post-LEEP and did not recur. A boundary marking tool was used to demarcate the squamocolumnar junction and any visible lesion(s) on digitized images; these areas were then converted to pixels and quantitated (19).

Pathology. In ALTS, clinical management was based on the clinical center pathologists' interpretation of cytologic and histologic specimens. The pathology quality control group reviewed most Thin Prep cytology and all histology specimens. The primary end point of post-LEEP detection of CIN2+ (recognizing that this subsumes residual, recurrent, and possibly incident disease), was defined as histologic diagnosis of CIN2+ from the clinical center. A more rigorous end point of post-treatment histologic CIN3/carcinoma (CIN3+), defined by the pathology quality control group, was also considered.

Statistical Analysis. Six hundred eighty-six women underwent LEEP at either study enrollment or during the follow-up period; women whose first LEEP was at the exit visit were not considered because they lacked follow-up information. Women were excluded if (*a*) CIN2+ was not diagnosed by the clinical center on baseline histology (the initial colposcopy-directed biopsy or the first LEEP, n = 20), or (*b*) if the interval between colposcopy-directed biopsy and LEEP was >6 months (n = 56), due to the concern that the covariates might no longer be relevant. The remaining 610 women comprised the analytic population (Fig. 1).

Test results from the visit just before the LEEP or immediately preceding the LEEP at the same visit were defined as pre-LEEP; post-LEEP test results were obtained at the visit following the LEEP. The overall cumulative incidence of recurrent CIN2+ during the follow-up was calculated and then stratified based on the post-LEEP results from cytology, HC2, and PCR. PCR results were used to categorize women into one of five risk groups using the following hierarchical model: (*a*) positive for HPV16; (*b*) else positive for HPV18; (*c*) else positive for other carcinogenic HPVs; (*d*) else noncarcinogenic HPV positive; or (*e*) else HPV negative. Women with multiple infections were categorized into the highest category, thereby assuming that HPV16 infection was the most disease-relevant infection, followed by HPV18, etc.

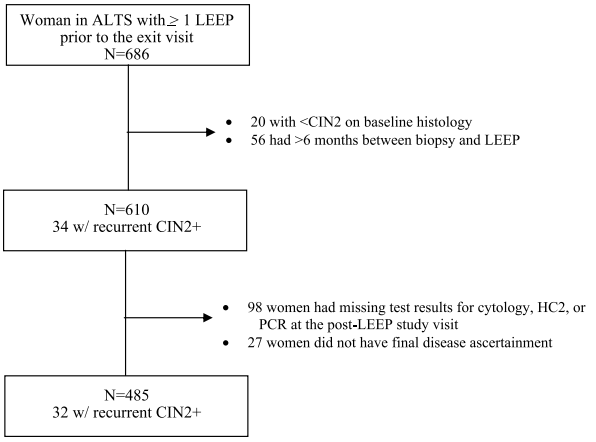

Figure 1. Composition of study population.

A modified Kaplan-Meier approach, where study visits were used instead of real time, was used to model the cumulative incidence of recurrent CIN2+ over time; the LEEP was considered ''time 0'' and each step of the KM curve was modeled based on the corresponding follow-up visit. This approach was chosen because follow-up visits clustered around 6-, 12-, 18-, and 24-month visits.

Of the 610 women, 98 were missing test results for either cytology, HC2, or PCR at the visit post-LEEP and 27 women did not have final disease ascertainment (i.e., either confirmed to be disease-free by colposcopy at exit or had recurrent CIN2+); these women were excluded from the comparative analysis of the clinical utility of the different testing methods. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each method for predicting recurrent CIN2+ during the study follow-up period were evaluated among the remaining 485 women. Post-LEEP testing methods included cytology [using three thresholds for test positivity: ASCUS or more severe (ASCUS+), LSIL or more severe (LSIL+), and HSIL or more severe (HSIL+)], HC2, PCR [four thresholds: carcinogenic HPV (restricted to the 13 types included in HC2), carcinogenic HPV persistence (defined as one or more of the same carcinogenic HPV type(s) present both pre- and post-LEEP), any HPV, and any HPV persistence], and cervigrams (one threshold: P1+). Combination testing, with referral based on positivity for either carcinogenic HPV or by cytology (with HPV-negative ASCUS not referred), was analyzed in two ways by using HC2 and PCR as the test for carcinogenic HPV. Repeated cytology (ASCUS+ threshold) twice post-LEEP (at approximate 6-month intervals) was also evaluated.

Using cytology (ASCUS+ threshold) as the referent group, McNemar's test (20) was used to evaluate if significant differences existed in the sensitivity and specificity (the exact test was used to evaluate sensitivity). Differences between tests in PPV and NPV were tested using the generalized estimating equation–based test method of Leisenring et al. (21), an analogue to the McNemar's test that conditions on the basis of test outcome instead of disease outcome. A χ^2 test was used to evaluate significant differences between referral percentages.

A receiver operating characteristic plot was created by graphing the sensitivity of detection of recurrent CIN2+ by the proportion of false-positives (100% − specificity) for each of the testing methods. Youden's J-statistic (J = sensitivity + specificity − 1; ref. 22), a summary index of the sensitivity and specificity, was calculated by applying equal weight to sensitivity and specificity, and then by overweighting the importance of sensitivity compared with specificity in a 2:1 ratio.

All P values reported are two-sided and considered statistically significant for $P < 0.05$.

Results

In ALTS, 610 women had histologic CIN2+ (51.1% had CIN2; 48.2% had CIN3) and underwent at least one LEEP during the study before the exit visit. The majority (70.0%) of these women had LEEP immediately following their enrollment study visit. Women had their subsequent visit a median of 4.5 months after LEEP (post-LEEP visit) and had a median overall follow-up time of 24 months.

Before LEEP (pre-LEEP), 89.9% [95% confidence interval (95% CI), 87.1-92.9] of women were positive for carcinogenic HPVs by PCR, whereas post-LEEP, 36.9% (95% CI, 32.7-41.1%) of women were positive. Of the women who were carcinogenic HPV-positive post-LEEP, 61.0% (95% CI, 54.0-67.6%) had at least one of the same carcinogenic HPV types present pre-LEEP, and were defined as having persistent HPV infection. At the post-LEEP visit, 33.7% (95% CI, 29.7-37.9%) had ASCUS+ cytology.

CIN2+ Recurrence. Following LEEP, 34 women were subsequently diagnosed with CIN2+ [median time to detection: 15.8 months (range 3.5-26.3 months)], 47.1% were detected during follow-up visits, and the remainder were detected at the exit visit. These women were demographically similar at the time of initial LEEP to women who did not later recur (Table 1). Compared with women who did not recur, women with recurrent CIN2+ more commonly had CIN3 baseline histology ($P = 0.01$), had larger lesions (measured by absolute number of LEEP tissue blocks containing CIN2+ by quality control pathologists; median 1 versus 2, $P = 0.01$), and differed by HPV positivity pre-LEEP ($P = 0.06$). There was no difference in the pre-LEEP presence of visible acetowhite lesions on the cervigram between women who had recurrent CIN2+ and those who did not (64.5 versus 71.9%, respectively, $P = 0.8$). However, compared with women who did not recur, women with recurrent CIN2+ were significantly more likely to have more than one lesion present on the pre-LEEP cervigram ($P = 0.04$).

Over the 2-year study period, the cumulative incidence of recurrent CIN2+ was 7% using Kaplan-Meier analysis (Fig. 2A). The results of cytology, HC2, and PCR at the visit post-LEEP were each evaluated for predicting recurrent CIN2+ over time. Stratifying women by cytology (negative versus ASCUS+ threshold for test positivity) or HC2 (negative versus positive) post-LEEP test results yielded similar cumulative incidence curves (Fig. 2A). However, using PCR data to define HPV risk categories further stratified risk of recurrent CIN2+. The 2-year absolute risk was highest for HPV16-positive women (37.0%, reference category) compared with the risk associated with HPV18 (18.5%, $P = 0.3$), other carcinogenic types (10.8%, $P < 0.001$), or noncarcinogenic types (1.5%; $P < 0.001$; Fig. 2B). Women who were HPV-negative post-LEEP had no recurrent disease during the follow-up period.

Test Performance at the Post-LEEP Visit for Detection of Recurrent CIN2+. Four hundred eighty-five of 610 women had test results from the post-LEEP visit and sufficient follow-up to ascertain final disease status (i.e., diagnosed with recurrent CIN2+ or confirmed disease-free by colposcopy at exit). Based on a threshold of ASCUS+, cytology was 78.1% (95% CI, 60.0-90.7%) sensitive and 69.1% (95% CI, 64.5-73.3%) specific for detecting recurrent CIN2+. Post-LEEP cytology was reported as ASCUS+ among 34.0% of women, resulting in a PPV of 15.2% (95% CI, 10.1-21.5%) and NPV of 97.8% (95% CI, 95.5-99.1%) for identifying women with recurrent histologic CIN2+. Using higher cytologic thresholds of LSIL+ and HSIL+ for referral to colposcopy resulted in significantly and considerably decreased sensitivity (Table 2).

Compared with cytology (ASCUS+ threshold), HC2 had nonsignificantly greater sensitivity (90.6%; 95% CI, 75.0-98.0%;

Table 1. Characteristics of women in ALTS who had CIN2+ baseline histology and LEEP, stratified by abscence versus presence of post-LEEP CIN2+ during follow-up

	No high-grade disease post-LEEP (n = 576) N (%)*	Recurrent CIN2+ post-LEEP (n = 34) N (%)*	P †
Study center			
Alabama	113 (93.4)	8 (6.6)	0.4
Oklahoma	158 (96.3)	6 (3.7)	
Pittsburgh	84 (96.6)	3 (3.4)	
Washington	221 (92.9)	17 (7.1)	
Study arm			
Immediate colposcopy	241 (92.0)	21 (8.0)	0.1
HPV triage	167 (96.5)	6 (3.5)	
Conservative management	168 (96.0)	7 (4.0)	
Age‡ (median, IQR)	24 (21-28)	24 (22-27)	0.6
Race			
White	422 (93.6)	29 (6.4)	0.1
Non-White	149 (96.7)	5 (3.3)	
Age at first vaginal intercourse (median, IQR)	16 (15-17)	16 (15-17)	0.4
No. sexual partners (median, IQR)	6 (4-11)	7 (4-14)	0.5
Oral contraceptive use‡			
Never	179 (93.2)	13 (6.8)	0.4
Ever	388 (94.9)	21 (5.1)	
Parity‡			
Nulliparous	257 (93.1)	19 (6.9)	0.3
One live birth	143 (96.6)	5 (3.4)	
Two or more live births	169 (94.4)	10 (5.6)	
Smoking‡			
Never smoker	223 (94.1)	14 (5.9)	0.2
Former smoker	75 (98.7)	1 (1.3)	
Current smoker	271 (93.5)	19 (6.5)	
Baseline histology			
CIN2	303 (97.1)	9 (2.9)	0.01
CIN3	269 (91.5)	25 (8.5)	
Cancer	4 (100)	0 (0)	
Pre-LEEP HC2			
Negative	18 (90.0)	2 (10.0)§	0.4
Positive	533 (94.7)	30 (5.3)	
Missing	25 (92.6)	2 (7.4)	
Pre-LEEP HPV type by PCR			
HPV16	281 (92.1)	24 (7.9)	0.06
HPV18	38 (100)	0 (0)	
Other carcinogenic HPV types	174 (96.7)	6 (3.3)	
Noncarcinogenic HPV types	31 (100)	0 (0)	
HPV negative	25 (89.3)	3 (10.7)§	
Missing	27 (96.4)	1 (3.6)	
Post-LEEP HC2			
Negative	319 (99.1)	3 (0.9)‖	<0.001
Positive	178 (86.0)	29 (14.0)	
Missing	79 (97.5)	2 (2.5)	
Post-LEEP HPV type by PCR			
HPV16	46 (69.7)	20 (30.3)	<0.001
HPV18	12 (85.7)	2 (14.3)	
Other carcinogenic HPV types	105 (91.3)	10 (8.7)	
Noncarcinogenic HPV types	106 (99.1)	1 (0.9)	
HPV negative	227 (100)	0 (0)	
Missing	80 (98.8)	1 (1.2)	

Abbreviation: IQR, intraquartile range.
*Unless otherwise noted.
†P value (two-sided) for Wilcoxon rank-sum test (medians) or χ^2 test (proportions) comparing abscence versus presence of recurrent CIN2+ during follow-up; Fisher's exact test was used for variables that had less than five women in a cell.
‡The variable was measured at the visit corresponding to the LEEP.
§Only one woman who had recurrent CIN2+ was negative pre-LEEP by both HC2 and PCR; two of three women who were PCR negative pre-LEEP was HC2 positive, and one of two women who were HC2-negative pre-LEEP was PCR positive.
‖The three women who had recurrent CIN2+ and were HC2 negative post-LEEP were HPV positive by PCR.

P = 0.3) and less specificity (63.8%; 95% CI, 59.2-68.2%; P = 0.048) for detection of recurrent CIN2+. The referral percentage, PPV, and NPV were similar to that of cytology (Table 2).

Carcinogenic PCR for the 13 HPV types tested for by HC2 had significantly greater sensitivity (96.9%; 95% CI, 83.8-99.9%; P = 0.03) and NPV (99.7%; 95% CI, 98.2-100%; P = 0.01) than cytology (Table 2); the specificity and PPV were not significantly different compared with cytology. One woman with recurrent CIN+ was not detected by carcinogenic HPV PCR; she was positive for HPV73 at the post-LEEP visit.

Combination testing with referral based on positivity for either carcinogenic HPV or cytology (with HPV-negative ASCUS not referred) was significantly more sensitive (96.9%; 95% CI, 83.8-99.9%; P = 0.03) and had greater NPV (99.7%; 95% CI, 98.1-100%; P = 0.02) than cytology alone; however, the referral percentage was significantly greater (41.0%, P = 0.02)

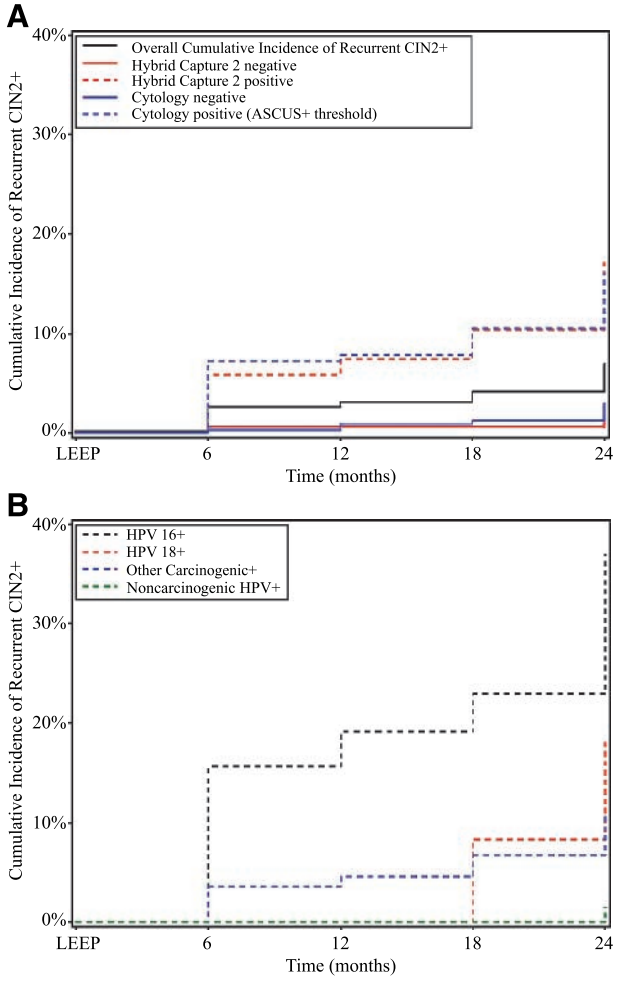

Figure 2. Cumulative incidence of post-LEEP recurrent CIN2+ at each 6-month study visit, stratified by results from the 6-month follow-up visit of cytology (ASCUS+ threshold), HC2, and HPV risk group from TS-PCR. **A.** *Black solid line,* overall cumulative incidence of recurrent CIN2+ in the study population; *blue and red lines,* cumulative incidence curve stratified by the results from cytology (ASCUS+ threshold) and HC2, respectively, at the visit 6 months post-LEEP; *dashed lines,* test-positive women; *solid lines,* test-negative women. The risk of recurrent CIN2+ was similar among cytology− and HC2-positive women. **B.** Risk categories based on HPV risk group using PCR results from the visit 6 months post-LEEP, which stratified the risk of recurrent CIN2+; the risk was considerably higher among HPV16-positive women even at the visit immediately following LEEP, whereas there was no risk of recurrent disease among HPV-negative women. Of note, the predictive values reported in Table 2 vary slightly from the stratified cumulative incidence curves because data presented in the graphs account for women who were censored or lost to follow-up.

Table 2. Estimated test performance for detection of post-treatment CIN2+ and percent of women referred based on different testing methods at the visit 6 months post-LEEP

	Referral, %	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Cytology*					
ASCUS+ †	34.0	78.1 (60.0-90.7)	69.1 (64.6-73.3)	15.2 (10.1-21.5)	97.8 (95.5-99.1)
LSIL+	*15.7*	*59.4 (40.6-76.3)*	**87.4 (84.0-90.3)**	**25.0 (15.8-36.3)**	96.3 (94.6-98.3)
HSIL+	*3.7*	*34.4 (18.6-53.2)*	**98.5 (96.8-99.4)**	**61.1 (35.7-82.7)**	*95.5 (93.2-97.2)*
HC2	39.8	90.6 (75.0-98.0)	*63.8 (59.2-68.2)*	15.0 (10.3-20.9)	99.0 (97.0-99.8)
HPV PCR					
Carcinogenic HPV	37.1	**96.9 (83.8-99.9)**	67.1 (62.6-71.4)	17.2 (12.0-23.5)	**99.7 (98.2-100)**
Carcinogenic HPV persistence ‡	*23.8*	77.4 (58.9-90.4)	**80.0 (75.9-83.7)**	**21.6 (14.4-30.4)**	98.0 (96.0-99.2)
HPV16	*12.8*	59.4 (40.6-76.3)	**90.5 (87.4-93.0)**	**30.6 (19.6-43.7)**	96.9 (94.8-98.4)
Any HPV	**57.5**	**100 (89.1-100)**	*45.4 (40.8-50.2)*	*11.5 (8.0-15.8)*	**100 (98.2-100)**
Any HPV persistence ‡	33.7	77.4 (58.9-90.4)	69.4 (64.9-73.7)	15.3 (10.0-21.9)	97.7 (95.4-99.1)
Combination testing §	**41.0**	**96.9 (83.8-99.9)**	*62.9 (58.3-67.4)*	15.6 (10.8-21.4)	**99.7 (98.1-100)**
Cervigram					
Low-grade impression (P1+)	*12.2*	*36.7 (19.9-56.1)*	**89.5 (86.2-92.2)**	19.3 (10.0-31.9)	*95.4 (92.9-97.2)*

*Each cytology threshold represents the finding of the specific cytologic abnormality or more severe, read by the clinical center pathologists, as the cut point for test positivity.

† Cytology (ASCUS or greater threshold) was the referent category for the comparisons between tests. Values in bold were significantly greater than the values for cytology at $P < 0.05$. Values in italics were significantly less than the values for cytology at $P < 0.05$.

‡ Persistence was defined as one or more of the same HPV types present both pre- and post-LEEP.

§ Referral based on positivity to carcinogenic HPV or cytology, with HPV-negative ASCUS not referred. When combined with cytology, using either HC2 or PCR as the test for carcinogenic HPV yielded exactly the same results.

and the specificity was significantly decreased (62.9%; 95% CI, 58.3-67.4%; $P = 0.02$). The results for combination testing were exactly the same whether HC2 or PCR were used as the test for carcinogenic HPV because all discordant HPV results were among LSIL+ women who were already considered test positive due to the cytology result.

Persistent carcinogenic HPV infection had similar sensitivity (77.4%, $P = 0.1$) but significantly higher specificity (80.0%, $P < 0.001$) and lower referral percent (23.8%, $P = 0.001$) compared with cytology. Repeating cytology twice after LEEP was 92.7% (95% CI, 83.2-100%) sensitive and 58.7% (95% CI, 54.1-63.3%) specific for identifying recurrent CIN2+; the referral percent was 44.7%.

On an receiver operating characteristic plot, methods that incorporated HPV detection, specifically PCR for carcinogenic types, HC2, and combination testing using HC2 and cytology, had high sensitivity without the considerable loss of specificity that was shown with PCR testing for any HPV type (Fig. 3). Youden's indices, both unweighted and weighted towards sensitivity, ranked TS-PCR for carcinogenic HPV highest, followed by combination testing using HC2 and cytology. HC2 alone ranked high using both indices, whereas a single cytologic assessment (ASCUS+ threshold) did not. The analyses of test performance were repeated using the more rigorous end point of post-LEEP CIN3+ as diagnosed by the pathology quality control group ($n = 18$) and the results were similar (data not shown).

Persistent HPV Infection: Recurrent versus Possible Incident CIN2+. *A priori*, all CIN2+ detected following LEEP was considered recurrent disease for the above analyses. However, of the 32 women with recurrent CIN2+ (and PCR data available), 5 (15.6%) had different HPV types pre-LEEP compared with the HPV types detected at recurrence. None of these five cases of possible incident CIN2+ was HPV16 positive post-LEEP and only one of five was CIN3+; by contrast, of the 27 cases with recurrent CIN2+ with at least one of the same HPV type(s) present at both time points, 17 (63.0%) were HPV16 positive and 18 (66.7%) were CIN3+.

Discussion

Following LEEP treatment, ~10% of women are found to have recurrent CIN2+ (2, 3). In our population of women with histologic CIN2+ who were treated by LEEP, the 2-year cumulative incidence of histologically confirmed, posttreatment CIN2+ was 7%. Because this risk of CIN2+ is much greater than in screening populations, post-LEEP surveillance strategies should emphasize sensitivity over specificity. Three surveillance testing methods, used at the visit following LEEP, maximized the sensitivity for detection of recurrent CIN2+, without undue loss of specificity: (*a*) PCR-based testing for 13 carcinogenic HPV types included in HC2, (*b*) combination testing using positivity to either carcinogenic HPV or cytology (excludes HPV-negative ASCUS), and (*c*) HC2 testing alone. Each method showed sensitivity over 90% for identifying women with recurrent CIN2+. Furthermore, the sensitivity using carcinogenic HPV PCR or combination testing was significantly greater than cytology alone (ASCUS+). However, cytology using a two-visit follow-up strategy can match the sensitivity of one carcinogenic HPV PCR test, but with an increase in the number of women referred to colposcopy and the potential for loss to follow-up. The sensitivity for HC2 alone was non-significantly greater than that of a single cytologic assessment; however, statistical power for estimating sensitivity was limited by the small number of cases ($n = 32$). Although PCR positivity for any HPV, which included types

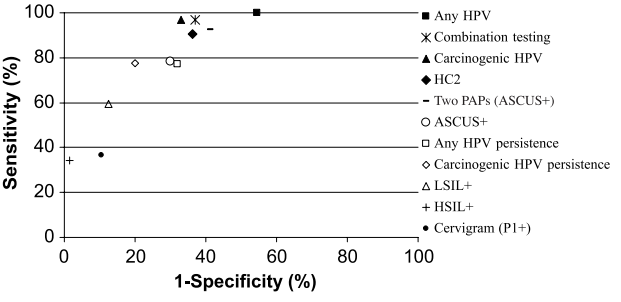

Figure 3. Receiver operating characteristic plot for different management strategies used at the post-LEEP visit to predict recurrent CIN2+. At the visit following LEEP, PCR for carcinogenic types, combination testing using positivity to carcinogenic HPV or cytology (with HPV-negative ASCUS not referred), and HC2 testing, each maximized sensitivity for detection of recurrent CIN2+ without undue loss of specificity.

that are not carcinogenic, had 100% sensitivity, this was not significantly different from the methods mentioned above, yet the specificity was significantly lower and the referral percent was significantly higher than for any of the other test methods considered.

Of the three testing methods that maximized the sensitivity, the specificity was highest for carcinogenic HPV PCR (67.1%) and was lower for combination testing and HC2 alone (~63%). A single ASCUS+ cytology had similar specificity to carcinogenic HPV PCR testing and was significantly more specific than combination testing and HC2 alone. Repeating cytology (ASCUS+ threshold) twice following LEEP had lower specificity than the above testing methodologies.

The PPV of each of these testing methods for detection of recurrent CIN2+ in the 2-year follow-up was not high (~15%). This was, in part, expected from the low prevalence of recurrent disease as well as the relatively short duration of follow-up. However, when the HPV PCR results from the post-LEEP visit were used to further stratify women by risk group, the cumulative incidence (or PPV) for recurrent CIN2+ differed even among carcinogenic HPV types. HPV16 positivity in samples collected 6 months post-LEEP enriched the 2-year absolute risk of CIN2+ to 37.0%, twice that of HPV18 (18.5%), and more than three times that of other oncogenic types (10.8%). The risk among carcinogenic HPV-negative women was 1.5%. The increased risk of cervical disease associated with HPV16 compared with other carcinogenic HPV types has been shown both in a normal screening population and in a triage context (23-25). In these settings, the risk of CIN2+ associated with HPV16 was ~2 to 2.5 times higher compared with that of other oncogenic types. Although it seems that posttreatment management strategies could similarly benefit from adjunctive HPV16 testing to further differentiate women at highest risk of disease recurrence, the sensitivity of HPV16 positivity alone was low (59.4%) and therefore could not be used as a sole strategy for surveillance. Similarly, cervicography, cytology using LSIL or greater for test positivity, and persistent HPV infection (either for carcinogenic or any HPV types) each had inadequate sensitivity and could therefore not be used as independent testing methods.

Sixty-three percent of women tested negative for carcinogenic HPV types by PCR at the visit post-LEEP. The NPV was very high (>99%) for PCR for carcinogenic HPVs and combination testing using HC2 and cytology; this was significantly higher than the NPV for cytology alone (ASCUS+; 97.8%). In this posttreatment population, high NPV is important due to the risk associated with returning women who test negative to a normal screening schedule.

For the purposes of this study, any disease that was CIN2+ following LEEP was considered ''recurrent'' disease. In the majority of cases, women had at least one of the same HPV type present pre-LEEP and at the time of recurrent CIN2+ diagnosis; most of the recurrent disease was actually CIN3 (63.0%). However, in 15% of women, ''recurrent'' CIN2+ was associated with different HPV type(s) post-LEEP compared with pretreatment, less severe disease (mostly CIN2), and no HPV16 positivity. We speculate that these cases represent incident CIN2+ and not post-LEEP recurrence.

There are several strengths of these data, including the size and prospective nature of ALTS, high rates of follow-up at 6-month intervals, rigorous pathology review, nearly complete ascertainment of final disease diagnosis, and dual HPV testing using HC2 and PCR. However, women in ALTS were recruited from the community for an interpretation of equivocal or low-grade cytology (and not HSIL). As a result, the underlying CIN2 and/or CIN3 lesions were smaller and therefore probably associated with less risk of recurrence compared with the general LEEP population (which includes women referred for a cytologic interpretation of HSIL; ref. 26). The small number of women with recurrent disease compromised the power to assess significant differences between tests and limited our ability to specifically examine the more rigorous disease end point of post-LEEP CIN3+. Additionally, we evaluated the tests at the 6-month visit post-LEEP; increasing the interval between LEEP and follow-up would likely have resulted in less carcinogenic HPV positivity post-LEEP, which would have in turn increased the PPV. Finally, as women remain at high risk for CIN2+ several years after treatment (27), follow-up beyond 2 years may have detected additional posttreatment CIN2+, but also more incident disease.

Our results show that a test for carcinogenic HPV types at the visit following LEEP, either alone or in combination with cytology, provides 97% sensitivity for detection of posttreatment CIN2+ while referring ~37% to 41% of women again to colposcopy. This exceeds the performance of HPV testing in ALTS for the triage of ASCUS, where sensitivity for detection of CIN3 was 72.3% with referral of 55.6% of women (15). Compared with the HPV-based methods, a single cytologic assessment (at the ASCUS+ threshold) post-LEEP was significantly less sensitive. Cytology repeated twice after LEEP had similar sensitivity to the HPV-based testing methods, but at the expense of the other measures of test performance. The best management strategy for women who, after LEEP, test carcinogenic HPV positive yet are negative by colposcopy requires further investigation. Nonetheless, integrating carcinogenic HPV testing into post-LEEP surveillance will likely increase the sensitivity for detection of posttreatment high-grade disease.

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